Differentiation potential of agouti (*Dasyprocta prymnolopha*) bone marrow mesenchymal stem cells before and after thwaning

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Abstract

The agouti (*Dasyprocta prymnolopha*) is a rodent that lives in South America. The isolation of mesenchymal stem cells from this species is a novelty in the field of wild animal medicine. The process of conservation and cryopreservation of mesenchymal stem cells is still a matter of study. This study aimed to isolate, expand and evaluate the differentiation potential of mesenchymal stem cells from agouti bone marrow mesenchymal stem cells (ag-BMMSC), before and after cryopreservation. Four adult agoutis were used for this purpose. These animals were maintained in captivity in the Nucleus for Studies and Wild Animal Preservation (NEPAS) from Federal University of Piauí (UFPI). Bone marrow aspirates from the femur were collected and delivered for the Stem Cell Laboratory (LABCelt) of the Integrated Nucleus of Morphology and Stem Cell Research (NUPCelt) for isolation and expansion of undifferentiated cells. Samples of cryopreserved cells for 36 months were expanded to achieve $1x10^6$ cells. The protocols for adipogenic and osteogenic differentiation were performed for 21 days in non-cryopreserved and cryopreserved cells and subsequently were fixed with paraformaldehyde for the performance of specific staining protocols using oil red (Sigma-Aldrich®) for adipogenic (Sigma-Aldrich®) and alizarin red for evaluate the cell differentiation. The medium used and protocols for differentiation as the same of this manufacturer (StemPro®). The cells were observed in inverted light microscope and the images were recorded for evaluation. Cells with fibroblastoid morphology and colony forming units (CFU) were observed. Furthermore, the cell proliferation in cryopreserved cells was slower than non-cryopreserved cells. The adipogenic differentiation of cryopreserved and non-cryopreserved cells showed several lipid vacuoles stained with Oil Red staining (Sigma-Aldrich®). From osteogenic differentiation were observed calcium deposits stained in brown-red color and the formation of nodule-like structures. The same characteristics were observed in cryopreserved cells, although in small number. Morphological alterations were also observed in 15 days. Some cell cultures maintained the fibroblastoid morphology even after differentiation. The isolation and expansion of ag-BMMSC was possible even after cry-preservation for 36 months. The morphology comparative study of non-cryopreserved and cryopreserved after

adipogenic and osteogenic differentiation showed positive results in both cases, however, with more cells stained in non-cryopreserved cells.

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